

Response of Chemostat Grown
Enteric Bacteria to Chlorine Dioxide

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The Response of Chemostat Grown
Enteric Bacteria to Chlorine Dioxide

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ABSTRACT

The effect of antecedent growth conditions on the sensitivity of Klebsiella pneumoniae and Yersinia enterocolitica was investigated. The two organisms were grown in a defined medium supplemented with glucose either in the chemostat or in batch culture, and the influence of growth rate, temperature and cell density on the sensitivity was studied. All inactivation experiments were conducted using a dose of 0.25 mg/l chlorine dioxide in a phosphate buffer saline at pH 7.0 and 23°C. Populations grown under conditions that resemble natural aquatic environments, e.g., low temperatures and growth at submaximal rates caused by nutrient limitation, were more resistant than those grown at higher temperatures and maximal rates as in batch culture. The conclusion from this study is that antecedent growth conditions have a profound effect on the susceptibility of bacteria to antimicrobial agents and that batch grown bacteria may not provide a true indication of the sensitivity of natural populations to these agents.

We have previously demonstrated that growth of Escherichia coli at submaximal rates due to nutrient limitation and relatively low temperatures greatly increases its resistance to chlorine dioxide and phenylphol (1,2). We were interested in investigating whether other organisms behave in a similar way to E. coli or not. For this reason, we decided to extend this study to include two pathogens of considerable concern to public health, Klebsiella pneumoniae and Yersinia enterocolitica.

K. pneumoniae and Y. enterocolitica were grown in a glucose mineral salt medium. Populations were grown either in a chemostat (Bio Flo, New Brunswick Scientific) or in batch culture. Dose-response experiments indicated that a dose of 0.25 mg/l ClO_2 , resulted in significant extents of kill while providing measurable concentrations of residual disinfectant (0.12 mg/l), and surviving organisms. Disinfection experiments were conducted in a 1 liter well-mixed pyrex flask, held at 23 ± 1 . Samples were withdrawn from the reactor and were mixed with $\text{Na}_2\text{S}_2\text{O}_3$ to neutralize any residual disinfectant. Samples were suitably diluted and filtered in triplicate through Gelman-GN-6 membrane filters. The filters were placed on M-Endo agar plates (Difco) and incubated at 25°C for 72 h in the case of Y. enterocolitica and at 37°C for 24 h in the case of K. pneumoniae.

The resistance was presented in terms of survival ratio (N_t/N_0) after t min of contact with chlorine dioxide.

The effect of D on sensitivity was tested at two different temperatures (15 and 29°C) in the case of Y. enterocolitica and at three temperatures in the case of K. pneumoniae (15, 25, and 37°C);

Y. enterocolitica did not grow at 37°C in the medium used. The plots show the resistant fraction (N_{15}/N_0) as a function of D or growth temperature. At a given temperature, the culture resistance to chlorine dioxide increased with decreasing D.

At a given dilution rate, temperature determined the extent of sensitivity of K. pneumoniae, and an increase in resistance occurred at low temperatures in the case of K. pneumoniae (Fig. 1). Temperature had only a marginal effect on the sensitivity of Y. enterocolitica to chlorine dioxide.

We have shown previously that culture cell density influences the sensitivity of E. coli to chlorine dioxide, a higher culture cell density during growth being accompanied with greater resistance. Studying the effects of cell density on the sensitivity is important because organisms in most aquatic environments grow at low cell densities compared to the laboratory batch cultures. Y. enterocolitica cultivated at a fixed D in a chemostat at two different glucose concentrations in the inflow medium (which determine the steady-state culture density) did exhibit differences in sensitivity, the lower density population being more resistant. K. pneumoniae, on the other hand, exhibited the same sensitivity after growth at two different culture densities.

The results clearly demonstrate that differences in sensitivity of microorganisms to antimicrobial agents could be attributed to their different antecedent growth conditions and that slowly growing populations of Y. enterocolitica and

K. pneumoniae are more resistant to disinfection by chlorine dioxide than populations grown at more rapid rates.

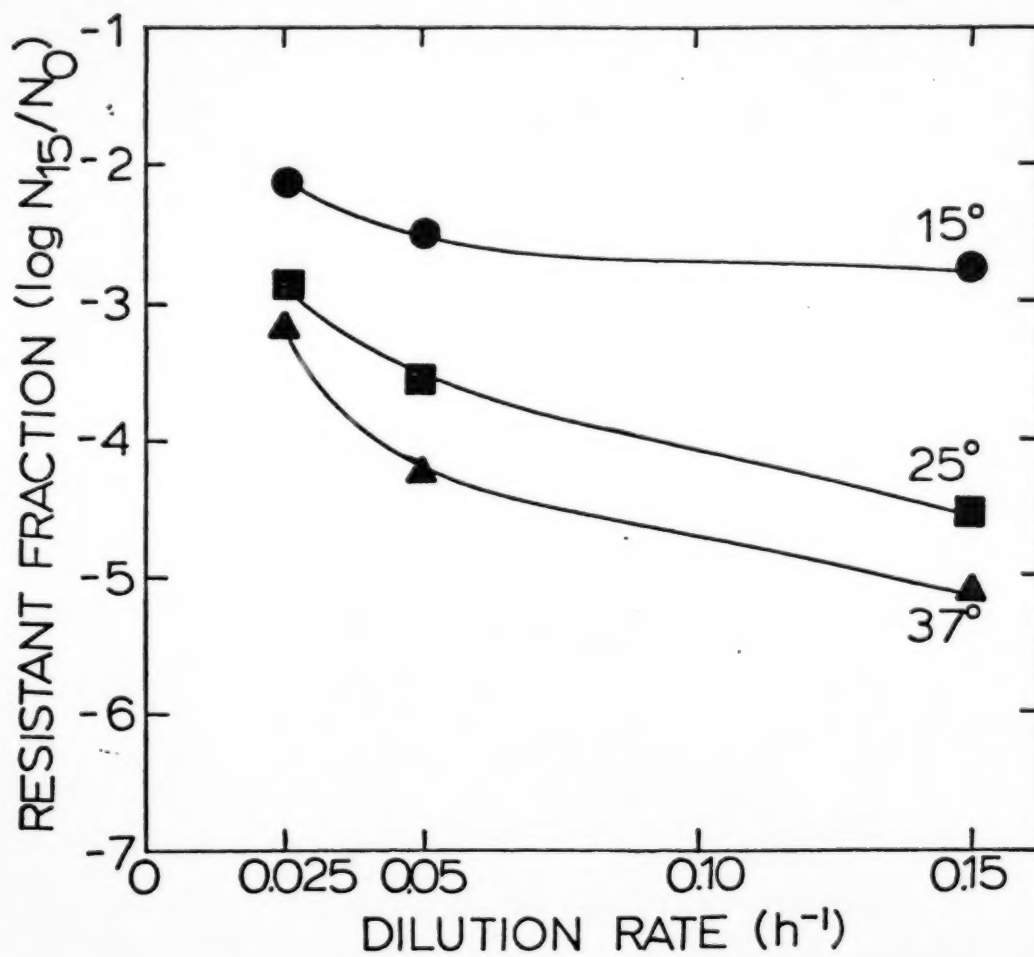
A comparison of the sensitivity patterns of the two microorganisms indicates that Y. enterocolitica was more sensitive to chlorine dioxide than K. pneumoniae. For instance, at 15°C and $D = 0.025 \text{ h}^{-1}$, treatment of Y. enterocolitica with chlorine dioxide produced a 5 log reduction, whereas under the same experimental conditions, K. pneumoniae showed a 2 log reduction.

In conclusion, populations grown under conditions that more closely approximate the nature environment are more resistant than those grown under conventional batch culture conditions so that the sensitivity of batch culture grown cells to antimicrobials of at least these organisms is not a suitable index of the sensitivity of natural populations. A detailed version of this manuscript has been submitted to Applied and Environmental Microbiology.

Fig. 1

Effect of dilution rate on sensitivity of Klebsiella pneumoniae to chlorine dioxide grown under glucose limitation at three temperatures. The concentration of glucose in the inflow medium was 0.4%.

Symbols: ● 15°C growth temperature,
■ 25°C growth temperature, and
▲ 37°C growth temperature.



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